

WHAT IS CLAIMED IS:

1. A microfluidic device, the device comprising:
- 5 (i) a body structure with a plurality of microscale channels disposed therein, the plurality of microscale channels comprising:
- (a) a deep mixing channel; and,
- (b) a shallow separation channel fluidly coupled to the deep mixing channel, wherein the deep mixing channel has a first cross-sectional area and the shallow separation channel has a second cross-sectional area, which first cross-sectional area is larger than the second cross-sectional area;
- 10 (ii) a pressure source in fluid communication with the deep mixing channel, which pressure source introduces one or more samples into the deep mixing channel by applying pressure to the deep mixing channel; and,
- 15 (iii) an electrokinetic controller in fluid communication with the shallow separation channel, which electrokinetic controller transports the one or more samples through the shallow separation channel by applying a voltage to the shallow separation channel
- 20 2. The microfluidic device of claim 1, wherein the deep mixing channel has a depth and a width, which depth is between about 5  $\mu\text{m}$  and about 100  $\mu\text{m}$  and which width is between about 5  $\mu\text{m}$  and about 100  $\mu\text{m}$ .
3. The microfluidic device of claim 2, wherein the depth is between about 10  $\mu\text{m}$  and about 50  $\mu\text{m}$  and the width is between about 20  $\mu\text{m}$  and about 50  $\mu\text{m}$ .
- 25 4. The microfluidic device of claim 3, wherein the depth is between about 10  $\mu\text{m}$  and about 20  $\mu\text{m}$  and the width is between about 35  $\mu\text{m}$  and about 45  $\mu\text{m}$
5. The microfluidic device of claim 4, wherein the depth is about 15  $\mu\text{m}$  and the width is about 40  $\mu\text{m}$ .

6. The microfluidic device of claim 1, wherein the shallow separation

channel has a depth and a width, which depth is between about 1  $\mu\text{m}$  and about 20  $\mu\text{m}$  and which width is between about 1  $\mu\text{m}$  and about 20  $\mu\text{m}$ .

7. The microfluidic device of claim 6, wherein the depth is between about 1  $\mu\text{m}$  and about 15  $\mu\text{m}$  and the width is between about 5  $\mu\text{m}$  and about 15  $\mu\text{m}$ .

5 8. The microfluidic device of claim 7, wherein the depth is between about 3  $\mu\text{m}$  and about 10  $\mu\text{m}$  and the width is between about 5  $\mu\text{m}$  and about 10  $\mu\text{m}$ .

9. The microfluidic device of claim 8, wherein the depth is about 3  $\mu\text{m}$  and the width is about 9  $\mu\text{m}$ .

10 ~~SWB~~ 10. The device of claim 1, wherein the deep mixing channel has a first depth and the shallow separation channel has a second depth, which first depth is at least about 2 times as deep as the second depth.

11. The device of claim 1, wherein the deep mixing channel has a first depth and the shallow separation channel has a second depth, which first depth is at least about 5 times as deep as the second depth.

15 12. The device of claim 1, wherein the deep mixing channel has a first depth and the shallow separation channel has a second depth, which first depth is at least about 10 times as deep as the second depth.

20 13. The device of claim 1, wherein the deep mixing channel has a first width and the shallow separation channel has a second width, which first width is at least about 2 times as wide as the second width.

14. The device of claim 1, wherein the deep mixing channel has a first width and the shallow separation channel has a second width, which first width is at least about 4 to about 5 times as wide as the second width.

25 15. The device of claim 1, wherein the deep mixing channel has a first width and the shallow separation channel has a second width, which first width is at least about 10 times as wide as the second width.

16. The microfluidic device of claim 1, wherein the shallow separation channel comprises a separation matrix.

17. The microfluidic device of claim 16, wherein the separation matrix comprises polyacrylamide, linear polyacrylamide, cross-linked polyacrylamide, non cross-linked polyacrylamide, polydimethylacrylamide, agarose, cellulose, or polydimethylacrylamide/co-acrylic acid.

~~Sub 3~~ 18. The microfluidic device of claim 1, further comprising a shallow loading channel fluidly coupled to the deep mixing channel and intersecting the shallow separation channel.

10 19. The microfluidic device of claim 18, wherein the shallow loading channel has a depth and a width, which depth is between about 1  $\mu\text{m}$  and about 20  $\mu\text{m}$  and which width is between about 1  $\mu\text{m}$  and about 20  $\mu\text{m}$ .

20. The microfluidic device of claim 19, wherein the depth is between about 1  $\mu\text{m}$  and about 15  $\mu\text{m}$  and the width is between about 5  $\mu\text{m}$  and about 15  $\mu\text{m}$ .

15 21. The microfluidic device of claim 20, wherein the depth is between about 3  $\mu\text{m}$  and about 10  $\mu\text{m}$  and the width is between about 5  $\mu\text{m}$  and about 10  $\mu\text{m}$

22. The microfluidic device of claim 21, wherein the depth is about 3  $\mu\text{m}$  and the width is about 9  $\mu\text{m}$ .

20 23. The microfluidic device of claim 1, wherein the pressure source applies a positive pressure or a negative pressure.

24. The microfluidic device of claim 1, wherein the pressure source comprises a vacuum.

~~Sub 3~~ 25. The microfluidic device of claim 1, wherein the pressure source comprises an electroosmotic pump fluidly coupled to the deep mixing channel.

25 26. The microfluidic device of claim 25, wherein the electroosmotic pump comprises a channel comprising a fluidic material, which fluidic material comprises a salt.

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27. The microfluidic device of claim 25, wherein the electroosmotic pump draws a sample into the deep mixing channel and the electrokinetic controller injects the sample from the deep mixing channel into the shallow separation channel.

28. The microfluidic device of claim 1, the device further comprising a shallow loading channel, wherein the electrokinetic controller electrokinetically loads the sample into the shallow loading channel from the deep mixing channel and electrokinetically injects the sample into the shallow separation channel from the shallow loading channel.

29. A method of performing a separation in a microfluidic device, the method comprising:

- (i) flowing at least a first sample through a deep mixing channel by applying pressure to the first sample in the deep mixing channel, which first sample comprises one or more components;
- (ii) flowing the first sample into a shallow separation channel by applying an electrokinetic force to the first sample, which shallow separation channel is fluidly coupled to the deep mixing channel; and,
- (iii) electrokinetically separating at least two of the one or more components of the first sample in the shallow separation channel, thereby performing a separation.

30. The method of claim 29, further comprising introducing a second sample into the deep mixing channel concurrent with electrokinetically separating the first sample.

31. The method of claim 29, further comprising electrokinetically loading the first sample from the deep mixing channel into a shallow loading channel and electrokinetically injecting the first sample from the shallow loading channel into the shallow separation channel, wherein the shallow loading channel is fluidly coupled to the deep mixing channel and intersects the shallow separation channel.

32. The method of claim 31, comprising providing the shallow loading channel to have a depth between about 1  $\mu\text{m}$  and about 20  $\mu\text{m}$  and a width between about 1

µm and about 20 µm.

33. The method of claim 32, comprising providing the shallow loading channel to have a depth between about 1 µm and about 15 µm and a width between about 5 µm and about 15 µm.

5 34. The method of claim 33, comprising providing the shallow loading channel to have a depth between about 3 µm and about 10 µm and a width between about 5 µm and about 10 µm

35. The method of claim 34, comprising providing the shallow loading channel to have a depth of about 3 µm and a width of about 9 µm.

10 36. The method of claim 31, further comprising introducing a second sample into the deep mixing channel concurrent with loading the first sample into the shallow loading channel.

37. The method of claim 36, further comprising applying substantially reduced pressure or no pressure during the separating step.

15 38. The method of claim 29, further comprising reacting at least the first sample with one or more reagents in the deep mixing channel, resulting in at least a first reacted sample, which first reacted sample comprises one or more components.

20 39. The method of claim 38, comprising providing the first sample to comprise a substrate and the one or more reagents to comprise an enzyme, and reacting the substrate and enzyme to produce a product.

40. The method of claim 29, comprising providing the deep mixing channel to have a first cross-sectional area and the shallow separation channel to have a second cross-sectional area, wherein the first cross-sectional area is greater than the second cross-sectional area.

25 41. The method of claim 29, comprising providing the deep mixing channel to have a depth between about 5 µm and about 100 µm and a width between about 5 µm and about 100 µm.

42. The method of claim 41, comprising providing the deep mixing channel to have a depth between about 10  $\mu\text{m}$  and about 50  $\mu\text{m}$  and a width between about 20  $\mu\text{m}$  and about 50  $\mu\text{m}$ .

43. The method of claim 42, comprising providing the deep mixing channel to have a depth between about 10  $\mu\text{m}$  and about 20  $\mu\text{m}$  and a width between about 35  $\mu\text{m}$  and about 45  $\mu\text{m}$ .

44. The method of claim 43, comprising providing the deep mixing channel to have a depth of about 15  $\mu\text{m}$  and a width of about 40  $\mu\text{m}$ .

45. The method of claim 29, comprising providing the shallow separation channel to have a depth between about 1  $\mu\text{m}$  and about 20  $\mu\text{m}$  and a width between about 1  $\mu\text{m}$  and about 20  $\mu\text{m}$ .

46. The method of claim 45, comprising providing the shallow separation channel to have a depth between about 1  $\mu\text{m}$  and about 15  $\mu\text{m}$  and a width between about 5  $\mu\text{m}$  and about 15  $\mu\text{m}$ .

47. The method of claim 46, comprising providing the shallow separation channel to have a depth between about 3  $\mu\text{m}$  and about 10  $\mu\text{m}$  and a width between about 5  $\mu\text{m}$  and about 10  $\mu\text{m}$ .

48. The method of claim 47, comprising providing the shallow separation channel to have a depth of about 3  $\mu\text{m}$  and a width of about 9  $\mu\text{m}$ .

49. The method of claim 29, comprising providing the deep mixing channel to have a first depth and the shallow separation channel to have a second depth, which first depth is at least about 2 times as deep as the second depth.

50. The method of claim 29, comprising providing the deep mixing channel to have a first depth and the shallow separation channel to have a second depth, which first depth is at least about 4 to about 5 times as deep as the second depth.

51. The method of claim 29, comprising providing the deep mixing channel to have a first depth and the shallow separation channel to have a second depth, which first

depth is at least about 10 times as deep as the second depth.

**52.** The method of claim **29**, comprising providing the shallow separation channel to comprise a separation matrix..

5 **53.** The method of claim **52**, wherein the separation matrix comprises polyacrylamide, linear polyacrylamide, cross-linked polyacrylamide, non cross-linked polyacrylamide, polydimethylacrylamide, agarose, cellulose, or polydimethylacrylamide/co-acrylic acid.

10 **54.** The method of claim **29**, further comprising providing a pressure source operably coupled to the deep mixing channel, which pressure source introduces the first sample into the deep mixing channel.

**55.** The method of claim **54**, wherein the pressure source comprises a vacuum source..

**56.** The method of claim **54**, wherein the pressure source comprises an electroosmotic pump.

15 **57.** The method of claim **56**, wherein the electroosmotic pump comprises a channel comprising a fluidic material, which fluidic material comprises a salt.